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Short communication

Uneven distribution of microorganisms on the surface of field-grown cantaloupes

Shefali Dobhal [a], Guodong Zhang [b], Dhiraj Gautam [a], Jacqueline Fletcher [a], Li Maria Ma [a, *]

[a] National Institute for Microbial Forensics & Food and Agricultural Biosecurity, Department of Entomology and Plant Pathology, Oklahoma State University, 127 Noble Research Center, Stillwater, OK 74078, USA
[b] Center for Food Safety and Applied Nutrition, Food and Drug Administration, College Park, MD 20740, USA

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ABSTRACT

Cantaloupes have been implicated in a number of foodborne illness outbreaks due to contamination with human pathogens. However, we have limited understanding on the potential microbial contamination routes, especially in the production fields. We hypothesized that the soil upon which cantaloupe fruit rest can be a source of microbial contamination. Microbial populations on the surfaces of field-grown cantaloupes were enumerated and the effect of washing on the removal of microorganisms from the cantaloupe surfaces was evaluated. The microbial populations on the lower surface (in direct contact with soil) of the cantaloupes were significantly higher ($p < 0.05$), averaging 2.21 log CFU/cm^2 (aerobic bacteria); 1.62 log CFU/cm^2 (coliforms); and 2.02 log CFU/cm^2 (molds and yeasts), compared to those on the upper surface (exposed to the air). Washing significantly reduced only the populations of yeasts and molds on the lower surfaces of cantaloupes. Scanning electron micrographs showed more microbe-like bodies on the lower surface of cantaloupe than on the top. This study revealed an uneven distribution of microbial populations on the surfaces of field-grown cantaloupes, suggesting that direct contact with soil can be a major source of microbial contamination to fruits. Field production practices that minimize direct contact of cantaloupes with the soil may serve as a control strategy for ensuring a safer product. Also, more effective surface cleaning methods should be explored.

1. Introduction

There has been an increase in disease outbreaks caused by the contamination of fresh produce by human pathogens in the United States (CDC, 2013; Sivapalasingam, Friedman, Cohen, & Tauxe, 2004). The increased concern in the United States and throughout the world about produce-associated outbreaks has stimulated increased surveillance for selected foodborne pathogens. Most well characterized outbreaks have been caused by bacteria such as *Salmonella* spp., *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Shigella* spp., and viruses such as norovirus and Hepatitis A virus (Beuchat, 1996; CDC, 2013). Cantaloupes have been implicated in a number of foodborne illness outbreaks due to contamination with the human pathogens *Salmonella* and *L. monocytogenes* (CDC, 2011a, 2011b, 2012). In 2011, a multistate outbreak of listeriosis linked to contaminated cantaloupes caused 33 deaths and 147 infections involving five subtypes of *L. monocytogenes* (CDC, 2011a). Another multistate outbreak in 2012 was also linked to cantaloupes contaminated by *Salmonella* Typhimurium and *Salmonella* Newport (CDC, 2012). These outbreaks highlight the need for proper intervention strategies to minimize the risk of contamination.

There are many possible routes, from farm to fork, by which cantaloupes could become contaminated with foodborne pathogens. Potential direct or indirect contamination can result from contact with the contaminated soil (upon which cantaloupe fruits rest), manure, poor hygiene, contaminated irrigation water, improper cleaning and sanitization of the farm and processing equipment, and improper or ineffective washing prior to transport, packing, and fresh-cut processing (Bowen, Fry, Richards, & Beuchat, 2006; Brackett, 1992; Gagliardi, Millner, Lester, & Ingram, 2003; Ukuku & Sapers, 2001). During field production, soil may be one of the most important factors contributing to contamination events. Foodborne pathogens such as *Salmonella*, shigatoxin producing *E. coli* and *L. monocytogenes,* persist in the soil, even under harsh environmental conditions for long periods of time (Ingham et al., 2004; Islam et al., 2003, 2004). Fresh produce, such as

* Corresponding author. National Institute for Microbial Forensics & Food and Agricultural Biosecurity, Department of Entomology and Plant Pathology, Oklahoma State University, Stillwater 74078, USA. Tel.: +1 405 744 5290.
E-mail address: li.ma@okstate.edu (L.M. Ma).

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cantaloupe, that is in close proximity to the soil during production could be at risk of contamination from the soil, thus compromising the microbial quality of produce. A survey conducted by the U.S. Food and Drug Administration (FDA) on the environmental samples collected from the cantaloupe farms associated with a multistate outbreak of salmonellosis found that the production fields were primary and most likely source of contamination; soil and water samples tested positive for multiple *Salmonella* serovars similar to the outbreak strains (FDA, 2013).

In the United States after harvest cantaloupes are handled and packed different ways in each states. However, a washing step, usually consisting of chlorinated water, is generally used to reduce microbial loads on cantaloupe (Sapers, 2005; Zhang, Ma, Phelan, & Doyle, 2009). Studies have shown that this treatment could reduce the microbial counts on the cantaloupe surface (Fan, Annous, Keskinen, & Mattheis, 2009).

The objectives of this study were to investigate (i) the impact of direct contact with soil on the distribution of natural microflora on the surface of field-grown cantaloupes, and (ii) the effect of washing on removal of microflora from the surfaces of cantaloupe fruit.

2. Materials and methods

2.1. Field-grown cantaloupes

Whole cantaloupes (*Cucumis melo* L. var. *cantalupensis* Naudin) with no soft spots or visible molds and a similar extent of netting were harvested and purchased from a local organic farm in Central Oklahoma. At the time of collection, each cantaloupe was marked for their upper (facing the sky) and lower (in direct contact with soil) surfaces prior to packing. The cantaloupes were transported to the laboratory in individual sterile bags on ice and were processed immediately at room temperature (18–22 °C).

2.2. Preparation of sanitizing solution and washing treatment

Clorox®, a commercial bleach containing 5.25% sodium hypochlorite (NaOCl, Clorox Company, Oakland CA), was diluted with sterile water to a concentration of 200 ppm of sodium hypochlorite (pH = 6.5) (Ukuku, 2006). The cantaloupes were divided into two groups, each consisting of five fruits: one group was washed with chlorinated water and other (control) received no treatment. For washing treatment, fruits were submerged individually in 4 L chlorine solution in a sterile container, manually shaken by hand for 2 min to ensure complete submersion and then washed for 1 min with tap water. The washed cantaloupes were air-dried at room temperature before sampling and processed as described below.

2.3. Preparation of rind plugs and microbiological analysis

The upper and lower surfaces from the cantaloupes (both washed and unwashed) were cut with sterilized stainless steel blades to produce rind plugs (devoid of edible flesh) with an external rind surface area of 3 cm^2. The rind tissues were placed individually into a sterile stomacher bag containing 20 ml of phosphate buffered saline (PBS, Fischer scientific, Pittsburgh, PA), massaged and shaken by hand for 2 min. The surface wash was collected and serially diluted 10-fold in 0.1% sterile peptone water (BD, Becton Dickinson, Sparks, MD). The microbial populations were determined by spread plating 100 μl of the serial dilutions in duplicate on plate count agar (PCA; BBL, Becton Dickinson, Sparks, MD) for total aerobic bacteria (APC), violet red bile agar (VRBA; BBL) for total coliforms and potato dextrose agar (PDA; BBL) plates for yeasts and molds. PCA and VRBA plates were incubated at 35 °C for 24 h; and PDA plates at 28 °C for 2 days. The colonies were counted, averaged and calculated in terms of $\log_{10}$ CFU/ml and converted to $\log_{10}$ CFU/cm^2. The experiment was replicated three times.

2.4. Sample preparation and scanning electron microscopy

Samples were prepared for the scanning electron microscopy (SEM) following a protocol provided by the Oklahoma State University (OSU) Microscopy Facility. Briefly, 0.25 cm^2 disks were prepared from the outer rind of unwashed (control) cantaloupe (upper and lower surfaces) using a sterile scalpel; care was taken not to disrupt the netting present on the surface. Three replicate disks per surface area per cantaloupe were collected for the microscopic analysis. The rind pieces were stabilized and fixed with 2% gluteraldehyde for 2 h at room temperature; rinsed three times with 0.2 M cacodylate buffer (washing buffer) before fixation in 1% osmium tetroxide for 1 h at room temperature. After another rinse with wash buffer the disks were subjected to an ethanol dehydration series of 30%, 50%, 70%, 80%, 90%, 95%, and three final rinses in 100% ethanol followed by critical-point drying. They were coated with gold/palladium (Au/Pd) alloy for 2 min using a MED 010 sputtering device (Balzers Union, Blazers, Liechtenstein). Digital images were collected at different magnifications using the secondary electron-imaging mode of a scanning electron microscope Model Quanta 600 (FEI Corporation, Hillsboro, Oregon), operated at 15–20 kV.

2.5. Statistical analysis

All experiments were performed three times with five replicates in each treatment. Microbial data were converted to $\log_{10}$ CFU/ml, calculated for $\log_{10}$ CFU/cm^2 and analyzed for differences in response to treatments using the general linear model procedure of the Statistical Analysis software (SAS Institute, Inc., Cary, NC). Duncan's multiple range test was used to determine the significant differences among the means ($p < 0.05$).

3. Results

3.1. Distribution of microflora on the surfaces of field-grown cantaloupes

There was uneven distribution of microorganisms on the surfaces of field-grown cantaloupes. The total aerobic bacteria plate count (APC) on the upper side of cantaloupe ranged from a geometric mean of 1.15–1.21 log CFU/cm^2. The coliform bacterial levels ranged from 0.74–0.86 log CFU/cm^2. The geometric mean level for molds and yeasts were 1.22–1.36 log CFU/cm^2. In comparison, the lower side contained a significantly higher ($p < 0.05$) number of microorganisms than the upper portion in all three categories (total APC, total coliforms and molds and yeasts). The total aerobic bacteria plate count on the lower surface ranged from the geometric mean of 2.19–2.24 log CFU/cm^2. The coliform levels ranged from 1.47 – 1.69 log CFU/cm^2 and levels for molds and yeasts were 1.94–2.06 log CFU/cm^2.

3.2. Effect of washing on microbial populations of cantaloupes

The microbial populations on the upper and lower surfaces of cantaloupes are summarized in Table 1. Washing had no significant effect ($p \geq 0.05$) on the microbial populations on the upper surface of the cantaloupe. The population of total aerobic bacteria on the upper surface remained same as before wash, with a geometric mean from 1.17 to 1.31 log CFU/cm^2. A similar trend was observed in total count of coliforms, remaining constant from 0.61 to 0.87 log CFU/cm^2. There was also no significant effect of washing on